CLAIMS

1. A collection of labelled target DNA molecules which are exonuclease derivatives of double-stranded DNA.

2. A collection according to claim 1, wherein the DNA molecules are fluorescently labelled.

3. A collection according to claim 1 or claim 2, wherein the DNA molecules are labelled by incorporation of labelled nucleotides

4. A collection according to any preceding claim, wherein the double-stranded DNA is cDNA.

5. A collection according to claim 4, wherein the cDNA is globally amplified cDNA.

6. A collection according to either claim 4 or claim 5, wherein the labelled target DNA molecules are prepared from cDNA by a complexity reduction technique.

7. A collection according to claim 6, wherein the complexity reduction technique comprises a restriction digestion technique.

8. A collection according to claim 6, wherein the complexity reduction technique comprises a subtraction technique.

9. A collection according to claim 6, wherein the complexity reduction technique comprises a cDNA display technique.

10. A method of producing a collection of labelled target DNA molecules according to claim 1, comprising:

(i) subjecting double-stranded DNA molecules to exonuclease digestion to produce a collection of essentially single-stranded DNA molecules; and

(ii) labelling the single-stranded molecules.

11. A method according to claim 10, wherein the single-stranded molecules are labelled by the action of terminal transferase in the presence of labelled nucleotides.

12. A method of producing a collection of labelled target DNA molecules according to claim 1, comprising:
(i) treating double-stranded DNA to obtain a labelled double-stranded DNA population; and
(ii) effecting exonuclease digestion of the labelled population to produce a collection of essentially single-stranded labelled DNA molecules.

13. A method according to claim 12, wherein the labelled double-stranded DNA population is prepared by the action of terminal transferase in the presence of labelled nucleotides.

14. A method according to claim 12, wherein the labelled double-stranded DNA population is prepared by PCR in the presence of labelled nucleotides.

15. A method according to any one of claims 10 to 14, wherein the exonuclease digestion comprises partial digestion of both strands of the double-stranded DNA molecules.

16. A method according to any one of claims 10 to 14, wherein the exonuclease digestion comprises digestion of one strand of the double-stranded DNA molecules.

17. A method according to claim 16, wherein one strand of the double-stranded DNA molecules incorporates a restriction site, the molecules are treated with the appropriate restriction enzyme to produce double-stranded molecules having a sticky end, and the exonuclease digestion is effected from the blunt end of the double-stranded molecules.

18. A method according to any one of claims 16 to 17, wherein the exonuclease digestion comprises digestion of the 3'-5' strand of the double-stranded DNA molecules.

19. A method according to any one of claims 10 to 18, wherein the exonuclease digestion is effected using exonuclease III.

20. A method according to any of claims 10 to 19, wherein the double-stranded DNA is cDNA, or a derivative thereof.

21. A method according to claim 20, wherein the cDNA is global amplified cDNA.

22. A method according to claim 21, wherein the global amplified cDNA is prepared by the steps of:

a) preparing a global cDNA population representative of gene expression in a biological sample of interest from mRNA of the sample by using primers and limiting concentrations of nucleotides;

b) homopolymer tailing the global cDNA population; and

c) amplifying the tailed global cDNA population.

23. A method according to claim 22, wherein at least one of steps a) and b) is effected in the presence of an acetate buffer.

24. A method according to claim 23, wherein the acetate buffer comprises Tris acetate incorporating potassium acetate and/or magnesium acetate.

25. A method according to either claim 23 or claim 24, wherein step a) comprises reverse transcription of mRNA using primers capable of binding to the poly A tail of mRNA, the reverse transcription being performed in the presence of limiting concentrations of nucleotides.

26. A method according to claim 25, wherein the primers comprise a homopolymer T tract capable of binding to the poly A tail of mRNA.

27. A method according to any one of claims 23 to 26, wherein step a) is effected in the presence of a buffer comprising:

20-500 mM Tris pH 8.3;
10-300 mM KCl;
1-20 mM $MgCl_2$;
2-200 mM Tris Acetate pH 7.9;
5-500 mM Potassium Acetate;
1-10 mM Mg Acetate.

28. A method according to any one of claims 22 to 27, wherein the reaction mixture of step a) further comprises:

5-500 µg/ml Glycogen;
0.01-5 % NP-40;
0.02-10 u/ml RNase Inhibitor; and
70-80 µg/ml BSA.
2 –200 µM dNTPs;
0.01-100 µM oligonucleotide.

29. A method according to claim 28, wherein the reaction mixture comprises 0.1-5 µM oligonucleotide

30. A method according to any one of claims 23 to 29, wherein step b) comprises homopolymer tailing the global cDNA population, produced in step a), to produce a population of double-stranded DNA comprising both homopolymer A and homopolymer T tracts.

31. A method according to claim 30, wherein the homopolymer tailing is performed using terminal transferase.

32. A method according to any one of claims 23 to 31, wherein step b) is effected in the presence of a buffer comprising:

10-250 mM Tris pH 8.3;
5-150 mM KCl;
0.5-10 mM $MgCl_2$;
2-200 mM Tris Acetate pH 7.9;
5-500 mM Potassium Acetate; and
1-10 mM Mg Acetate.

33. A method according to any of claims 22 to 32, wherein step b) is performed in the presence of bovine serum albumen.

34. A method according to any of claims 22 to 33, wherein step b) is performed in the absence of DTT.

35. A method according to any of claims 22 to 34, wherein the reaction mixture of step b) comprises:
2.5-250 µg/ml Glycogen
0.005-2.5 % NP-40;
0.1-10 mM $CoCl_2$;
1 –100 µM dNTPs;
0.005-2500 µM dT24;
0.01-5 u/ml RNase Inhibitor;
35-40 µg/ml BSA;
0.05-5 mM additional dATP; and
1-500 u/ml TdT enzyme.

36. A method according to any of claims 22 to 35, wherein step b) is performed in the presence of 0.5-2 mM $CoCl_2$

37. A method according to claim 36, wherein step b) is performed in the presence of 1mM $CoCl_2$.

38. A method according to any one of claims 23 to 37, wherein step c) comprises amplifying the tailed double-stranded DNA by performing the polymerase chain reaction using the primers employed in step a).

39. A method according to any one of claims 23 to 38, wherein step c) is effected in the presence of a buffer comprising:

20-500 mM Tris pH 8.3;
10-300 mM KCl;
1-20 mM $MgCl_2$;
2-200 mM Tris Acetate pH 7.9;
5-500 mM Potassium Acetate; and
1-10 mM Mg Acetate.

40. A method according to any one of claims 22 to 39, wherein the reaction mixture of step c) comprises:

6-7 µM Oligonucleotide;
0.1-10 mM dNTPs;
2.5-250 µg/ml Glycogen;
0.03-3.3 mM $CoCl_2$;
0.02- 1% Triton X-100;
0.005-2.5 % NP-40;
35-40 µg/ml BSA;
0.05-5 mM additional dATP;
0.005 2500 µM dT24;
0.01-5 u/µl DNA Polymerase;
0.01-5 u/ml RNase Inhibitor; and
1-500 u/ml TdT enzyme.

41. A kit for the preparation of a collection of labelled target DNA molecules according to claim 1, the kit comprising:

(i) an exonuclease;
(ii) terminal transferase; and

(iii) labelled nucleotides.

42. A kit for the preparation of a collection of labelled target DNA molecules according to claim 1, the kit comprising:

(i) an exonuclease;

(ii) primers; and

(iii) labelled nucleotides.

43. A kit according to claim 42, further comprising reagents for PCR.

44. A kit according to any one of claims 41 to 43, wherein the labelled nucleotides are fluorescently labelled.

45. A kit according to any one of claims 41 to 44, wherein the exonuclease is exonuclease III.

46. A kit according to any one of claims 41 to 45, further comprising reagents for production of cDNA.

47. A kit according to claim 46 comprising reagents for production of global amplified cDNA.

48. A kit according to claim 47, wherein the reagents for production of global amplified cDNA comprise acetate buffers.

49. A kit for carrying out the method of any one of claims 27 to 40.

50. A kit according to claim 48 or 49, wherein the reagents for production of global amplified cDNA comprise:

Tris HCl;

KCl;

$MgCl_2$;

Tris acetate;

K acetate; and

Mg acetate.

51. A kit according to claim 50, including a first solution comprising:

20-500mM Tris HCl;

10-300mM KCl;

1-20mM $MgCl_2$;

2-200mM Tris acetate;

5-500mM K acetate; and

1-10mM Mg acetate.

52. A kit according to either claim 50 or claim 51, including a second solution comprising:

10-250 mM Tris pH 8.3;

5-150 mM KCl;

0.5-10 mM $MgCl_2$;

2-200 mM Tris Acetate pH 7.9;

5-500 mM Potassium Acetate; and

1-10 mM Mg Acetate.

53. A kit according to any one of claims 49 to 52, further comprising:

Glycogen;

$CoCl_2$;

NP-40;

dNTPs;

dT;

RNase inhibitors;

bovine serum albumen;

dATP; and

Triton X-100.

54. A kit according to claim 53 comprising:

2.5-250μM Glycogen;

0.1-10mM $CoCl_2$;

0.005-2.5% NP-40;

1-100μM dNTPs;

0.005-2500μM dT;

0.01-5u/ml RNase inhibitors;

70-80μg/ml bovine serum albumen;

0.05-5mM dATP; and

0.02-1% Triton X-100.

55. A kit according to any one of claims 41 to 54, further comprising reverse transcriptase.

56. A kit according to any one of claims 41 to 55, further comprising a DNA polymerase.

57. A kit according to any one of claims 42 to 56, further comprising terminal transferase.